

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re- application of MALLET et al

Serial No. 10/511,343

Group art Unit: 1633

Examiner: Fereydoun G. Sajjadi

Filed 04/11/2005

For: "Optimization of transgene expression in mammalian cells."

DECLARATION UNDER RULE 132

Hon. Commissioner of Patents and Trademarks
WASHINGTON D.C. 20231

Sir:

I, Jacques Mallet, residing at Paris, 18 rue Charcot 75013, France,

Declare and Say:

I am a citizen of France.

I am a Doctor in Science (PhD, Harvard University) and permanent researcher ("Directeur de Recherche, Classe Exceptionnelle") at the CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS).

I am an inventor of the present patent application (Serial No. 10/511,343).

I read the Barry *et al.* reference (Human Gene Therapy, 12:1103-1108, June 10, 2001). This document describes lentiviral vectors encoding a "Central Polypurine Tract" and a "Posttranscriptional Regulatory Element".

First, it is important to note that the Central Polypurine Tract (referred to as the cPPT element") is not a posttranscriptional regulatory element.

Barry *et al.* themselves indicate: "*The cPPT elements from the pol region of HIV-1 have been shown to act by increasing nuclear transport of the virus preintegration complex and hence increasing transduction efficiency (Charneau and Clavel, 1991; Follenzi et al.; 2000; Sirven et al., 2000; Zennou et al., 2000)*" (see page 1104, left column, lines 13-17 of Barry *et al.*).

As a first conclusion, the Barry et al. reference, contrary to the present invention, does not describe synergistic effects obtained by combining at least two distinct posttranscriptional regulatory elements.

In the enclosed Brun *et al.* reference (Molecular therapy vol. 7, no 6, June 2003), the authors of which are also inventors in the present application, **the effects of the four post transcriptional elements (WPRE, TH, APP and tau elements) alone and in combination, in plasmid vectors as well as in recombinant virus (lentiviral vectors), are described.**

The results of these experiments, using cell lines and primary cultures of rat cortical neurons, are summarized in Figures 3, 4 and 5 of Brun *et al.*.

Figure 5 (A) demonstrates that, the transduction of the above mentioned rat cortical neurons primary cultures with:

- a lentiviral vector containing the APP and WPRE elements increases by about 15 fold the luciferase expression.
- a lentiviral vector containing the APP, WPRE and Tau elements increases by about 30 fold the luciferase expression.

The abstract of Brun et al. further indicates that in "*neuronal cells, WPRE and both tau3'UTR and APP5' UTR had an additive effect on expression. The combination of the three elements increased the basal level of expression by up to 26-fold*".

It is further important to note that all the lentiviral vectors tested in Brun et al. (see page 788, paragraph entitled "Production of lentiviral vectors") contain the cPPT sequence (also called the flap sequence), as do the lentiviral vectors tested by Barry *et al.*.

The cPPT sequence increases the vector transduction efficacy by about 10 fold, due to the stimulation of the genome vector nuclear import [see Zennou *et al.*, 2001 (naming in particular Dr. Jacques Mallet as author), 2001, page 448, right column, and Zennou *et al.*, 2000, page 180, Figure 6B].

Considering that all the lentiviral vectors, including control vectors, tested in Brun et al. contain the cPPT sequence, the combined effect of the cPPT sequence and of at least two, for example three, post transcriptional elements, would be a 150 to 300 folds increase of the transgene expression, relative to the expression obtained using a control vector deprived of said cPPT sequence and post transcriptional elements.

In comparison, a 42-fold increased GFP expression is reported in Barry et al. using a GFP virus containing both a cPPT element and a PRE element relative to their control virus which deprived of the cPPT and the PRE sequences.

As a second conclusion, the Brun *et al.* reference thus demonstrates a considerably increased efficiency of the vectors according to the present invention when compared with the vectors of Barry *et al.*.

The Brun *et al.* reference further demonstrates that the present invention may be applied using different kind of vectors. It is further to be noted that results obtained in primary cultures of rat cortical neurons reflect the *in vivo* efficacy of the vectors according to the present invention, and of the compositions comprising said vectors.

As an additional comment, it is to be noted that the post transcriptional PRE element discussed in Barry et al, is of viral origin and is over 1000 bp long ("1161 bp", see page 1105, left column, line 18).

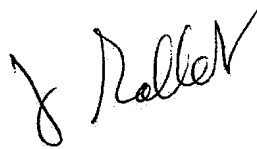
In considering the limited capacity (about 8000 bp) of lentiviral vectors (and of most other vectors) and the fact that the transduction efficiency of lentiviral vectors dramatically decreases with size, the usefulness of this sequence, in the context of our invention, is quite limited.

On the contrary, the posttranscriptional sequences we have used in the present invention are quite short: 609, 95, 237, and 91 bp for the WPRE (SEQ ID NO: 1), APP (SEQ ID NO: 2), tau (SEQ ID NO: 3) and TH (SEQ ID NO: 4) sequences, respectively.

Prior art does not suggest the vector according to the invention and does not suggest its effect in enhancing expression of the transgene.

The undersigned Declarant declares further that all statements made herein of this own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this day of January 24, 2008

A handwritten signature in black ink, appearing to read "J. Mallet", is written above the printed name.

JACQUES MALLET

CURRICULUM VITAE

Jacques MALLET

Born: 17 April 1945, Neuilly-sur-Seine, France

Current Address: LGN / CNRS UMR7091

Bâtiment CERVI, Hôpital de la Pitié Salpêtrière-

83 boulevard de l'Hôpital -75013 Paris, France

Tel 33 1 4217 7532 Mobile 33 6 0380 9499

Fax 33 1 4217 7533

jacques.mallet5@wanadoo.fr

Higher Education

1964-1967 Ecole Nationale Supérieure de Chimie de Montpellier, - Chemical Engineering

1967-1972 Harvard University (USA), Ph D in Physical Organic Chemistry

Experience

1967 – 1972 Graduate Studies: Laboratory of Professor Paul Bartlett, Department of Chemistry, Harvard University, Cambridge,

1972 – 1973 Military service: Department of Computer Science & Chemistry, University de Paris VII, Paris

1973 – 1980 Postdoctoral studies: Laboratory of Professor Changeux, Institut Pasteur, Paris

1980 – 1984 Responsable for Laboratoire de Neurobiologie, Institut de Microbiologie, Université de Paris-Sud XI, Orsay (creation of new laboratory)

1984-1992 Responsable for Département de Génétique Moléculaire, Laboratoire de Neurobiologie Cellulaire et Moléculaire du CNRS, UPR 0023, CNRS, Gif-sur-Yvette

1992-2000 Director, Unité Mixte de Recherche CNRS/Rhône-Poulenc/Aventis (UMR C 9923) Laboratoire de Génétique Moléculaire de la Neurotransmission et des Processus Neurodégénératifs (LGN)

2000-present Director, Unité Mixte de Recherche CNRS/Université Paris VI (UMR 7091) LGN

Titles

1968 Teaching Fellow, Department of Chemistry, Harvard University, Cambridge,

1970 Research Fellow, Department of Chemistry, Harvard University, Cambridge,

1973 DGRST Fellow, Institut Pasteur, Paris

1976	Chargé de Recherche, CR2, CNRS
1980	Chargé de Recherche, CR1, CNRS
1983	Directeur de Recherche, DR2, CNRS
1990	Directeur de Recherche, DR1, CNRS
2007	Directeur de Recherche, DRCE, CNRS

Honors and Awards:

Fullbright Fellowship, 1967-68

Member of EMBO since 1988

Member of Academia Europea since 1989

Corresponding Member of French Academy of Sciences: Field: "Cellular & Molecular Biology" since 1993

Member of European Academy of Sciences, Brussels, since 2003

Prize of the Fondation de Physiopathologie Lucien Dautrebande, Belgique, 1994

Prize of the French Atomic Energy Commission, 2000

Prize of Neurobiology from the Fondation pour la Recherche Médicale, 1983

Prize of Dr. and Madame Henri Labbé, Académie des Sciences, 1983

Visiting Professor, University of Jerusalem, of the "Lady Davies Fellowship Trust", December 1996-January 1997

Other informations

Has published more than 350 scientific articles and 85 review articles, and has applied/obtained 40 patents

Has trained more than 50 graduate students and numerous postgraduate fellows from North America, Asia and Europe

Has participated in the teaching of numerous Graduate Courses, as well as several EMBO and Cold Spring Harbor Courses

Has been invited to present lectures at international meetings (around 200) and have given seminars in the most prestigious universities and research centers in Europe, Japon and United States.

Coordinator of numerous contracts since 1992 including Human Frontiers Program, European Science Foundation, and the European Union

Professor "Ecole Polytechnique" 1987-1999

Consultant Aventis 1984-2004

Consultant NIMH, 1998,

Founding editor Neurobiology of Disease (1988) and has been or is a member of the editorial board of numerous scientific journals including EMBO Journal, Trends in Neurobiology, Current Opinions in Neurobiology

Member of Advisory Board of the International Institute of Molecular and Cell Biology, Warsaw, since 2003

Member of Advisory Board of the Riken Brain Science Institute, Tokyo, since 2004

Member of scientific council of several associations such as “France Alzheimer” and “Retina France”